

Mice Expressing Activated CaMKII Lack Low Frequency LTP and Do Not Form Stable Place Cells in the CA1 Region of the Hippocampus

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Summary

To relate different forms of synaptic plasticity to the formation and maintenance of place cells in the hippocampus, we have recorded place cells in freely behaving, transgenic mice that express a mutated Ca^{2+} -independent form of CaM Kinase II. These mice have normal long-term potentiation (LTP) at 100 Hz, but they lack LTP in response to stimulation at 5–10 Hz and are impaired on spatial memory tasks. In these transgenic mice, the place cells in the CA1 region have three important differences from those of wild types: they are less common, less precise, and less stable. These findings suggest that LTP in the 5–10 Hz range may be important for the maintenance of place-field stability and that this stability may be essential for the storage of spatial memory.

Introduction

Explicit memory storage is concerned with memories about places, objects and people (for review, see Squire, 1992; Schacter and Tulving, 1994). This type of memory storage requires the medial temporal lobe and, particularly, the hippocampus (Scoville and Milner, 1957; Olton and Werz, 1978). In rodents such as rats and mice, a major focus in the study of explicit memory storage has been on the storage of spatial information, or memory about place. Thus, there is evidence in rodents that the hippocampus is involved in the acquisition of environment-specific cognitive maps (O'Keefe and Nadel, 1978). A new map is learned for each new environment and can subsequently be used to solve spatial problems in that environment. The existence of such maps raises three questions: (1) How does an animal form a cognitive map in a new environment? (2) How is the map maintained stably over time? (3) and, How is the map used to solve spatial problems?

In the 1970s, two independent findings helped to shape modern thinking about the functioning of the hippocampus in spatial problem solving and spatial memory. First, the discovery by O'Keefe and Dostrovsky (1971) of hippocampal "place cells" led them to propose that the hippocampus can form cognitive maps. They found that spatial location is encoded in the pattern of

firing of individual hippocampal pyramidal cells. When an animal moves around a familiar environment, different place cells in the hippocampus fire as the animal enters different regions of space. A given cell fires only when the head of the animal is in a certain part of the environment called the "place field" or "firing field" of the cell. As the rat moves around, some cells start firing and others cease so that the firing of many cells signals the momentary location of the animal.

When the animal enters a new environment, new place cells form in a matter of minutes (Hill, 1978; Bostock et al., 1991; Wilson and McNaughton, 1993) and are stable for weeks or months in the new environment (Muller et al., 1987; Thompson and Best, 1989; Bostock et al., 1991). Hippocampal pyramidal cells can represent many environments without interference since only about half of the 10^6 pyramidal cells are place cells in a given environment. If this subset is randomly chosen for each environment, the number of possible maps is very large, and it is unlikely that any pair of maps will closely resemble each other.

In a second major finding of the early 1970s, Bliss and Lomo (1973) (and subsequently others) discovered that the major synaptic pathways in the hippocampus, including the Schaffer collateral pathway between the CA3 and CA1 regions, undergo a use-dependent form of synaptic strengthening now called long-term potentiation (LTP) (for review, see Bliss and Collingridge, 1993). The properties of LTP made it an excellent candidate for a hippocampally based storage mechanism for spatial (and other) forms of memory.

Despite the importance of LTP as a mechanism of plasticity and of place cells as a mechanism for generating a neural representation of space, there have been few systematic attempts to relate the two sets of findings to each other. As a result, we know little about the molecular mechanisms by which new place cells form, nor about how alterations in place-cell properties affect the behavior of the whole animal. For example, it is now clear that LTP is not a unitary phenomenon but a family of mechanisms for changing synaptic strength. Nevertheless, it is not known which (if any) component of LTP is important for the synaptic changes that are necessary for animals to store a new spatial representation.

To bridge the gap between place-cell biology and the molecular study of synaptic plasticity, we adapted place-cell recording methods to freely behaving mice and studied how genetic changes in synaptic plasticity affect place cells and how changes in place cells could be related to changes in behavior. Using this approach, we addressed three questions: (1) How are place cells formed? (2) Once formed, how are they maintained? and, (3) What do place cells contribute to the learning and performance of spatial problems?

To answer these questions, we examined place cells in a line of transgenic mice that expresses in the hippocampus a form of Ca^{2+} /calmodulin-dependent protein kinase (CaMKII) that was made Ca^{2+} -independent by a Thr-Asp mutation at position 286 (CaMKII-Asp286) (Mayford et al., 1995). These transgenic mice show normal high frequency LTP at 100 Hz. However, they have

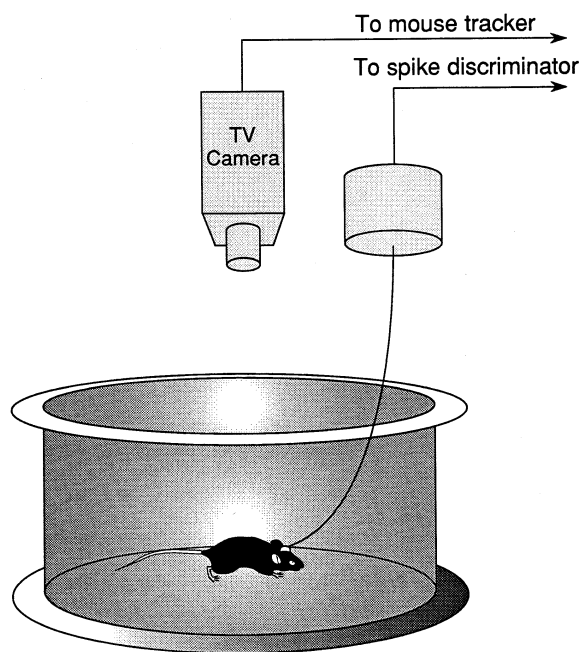


Figure 1. Diagram of the Recording Setup

A mouse is attached to a recording cable and placed inside a 49 cm diameter, 34 cm high cylinder. The other end of the cable goes to a 25-channel commutator whose fixed side is attached to a computer-based spike discrimination system. The cable is also used to supply power to a light-emitting diode on the headstage of the mouse. The entire apparatus is viewed with an overhead TV camera whose output goes to a tracking device that detects the position of the light in each 1/60 sec TV field. The output of the tracker is sent to the same computer used to detect spikes, so that parallel time series of positions and spikes are recorded. The occurrence of spikes as a function of position is extracted from the basic data and is used to form two-dimensional firing-rate patterns that can be numerically analyzed (see Experimental Procedures) or visualized as color-coded firing-rate maps (see Figures 3, 4, and 6).

a specific deficit in LTP when stimulation is in the 5–10 Hz range of frequencies, the frequency range of an endogenous oscillation in the hippocampal EEG known as the theta rhythm. These transgenic animals also show a severe deficit in spatial memory, although they retain the ability to perform nonspatial tasks (Bach et al., 1995).

In these CaMKII-Asp286 transgenic mice, place-cell activity shows three abnormalities. Place cells are less common; the place-cell firing fields that are formed are less precise; and once formed, the firing fields are less stable over time than in the wild-type mice. Together, these data suggest that interfering with LTP produced by activity in the range of theta frequency interferes with both the formation and stability of place cells. These effects can account for the deficits these mice show in spatial memory.

Results

We recorded action-potential firing from one or more individual hippocampal CA1 pyramidal cells in animals that were freely exploring a 49 cm diameter cylindrical chamber (Figure 1). A single orienting cue card covered 90° of the arc of the cylinder. The position of the animal

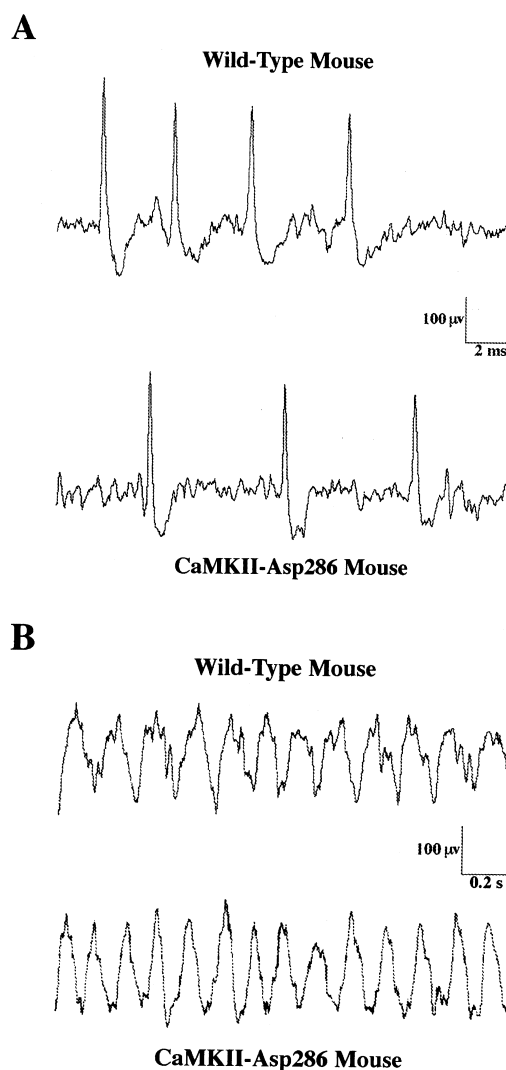


Figure 2. Firing Patterns and EEG in Wild-Type and Transgenic Hippocampal Neurons

(A) Complex-spike bursts recorded from a wild-type pyramidal cell and a transgenic pyramidal cell. Note the short interspike interval and the decreasing amplitude of the successive components.

(B) Recordings of the hippocampal EEG from a wild-type and a transgenic mouse. Note the difference in time scale from the scale used for the complex-spike examples. The rhythmic, sine-like pattern in each example is characteristic of the hippocampal EEG theta pattern, whose frequency range is from 5 to 10 Hz. The theta frequency is about 6.5 Hz for the wild-type example and about 7.5 Hz for the transgenic example. The data in this figure suggest that the transgenic mice are normal with regard to spike generation mechanisms and the gross features of the hippocampal EEG.

in the chamber was recorded simultaneously with the recording of neuronal firing. In this way, we could then measure the firing rate of each pyramidal cell as a function of the animal's head position within the cylinder.

The electrophysiological properties of unitary waveforms recorded from CA1 in wild-type and transgenic mice closely resemble those from rats (Figure 2A). The wild-type and CaMKII-Asp286 transgenic mice also show similar theta-frequency oscillation in the hippocampus (Figure 2B). To minimize sampling bias and to

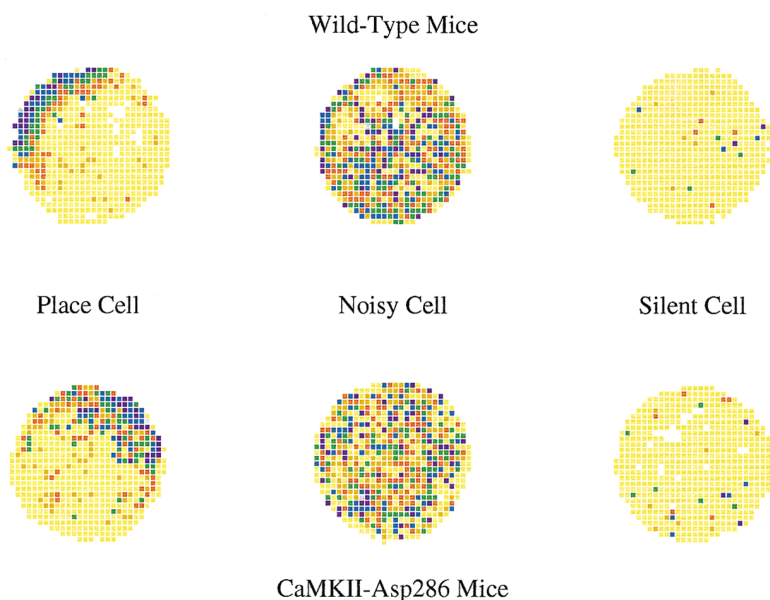


Figure 3. The Three Kinds of Positional Firing Patterns Characteristic of Place Cells, Noisy Cells, and Silent Cells

Cells of each type were seen in wild-type and transgenic mice. For place cells, firing is concentrated in 1 or 2 firing fields. The firing field in the wild-type place-cell example is the dark region at 10:00 o'clock; for the transgenic example, the field is at 1:30. Place cells in mice show lower peak firing rates than in rats. Silent cells are named for the fact that they discharge only a few spikes during a recording session. Noisy cells discharge at an appreciable rate (> 1.0 spike/sec) but show no tendency to fire in a restricted area. For wild-type mice, 9/26 cells were judged noisy and 2/26 cells silent. For transgenic mice, 25/52 cells were judged noisy and 11/52 silent. The proportions of noisy and silent cells are statistically equal in the two mouse types (noisy cells: $z = 1.13$, $p > 0.35$; silent cells: $z = 1.50$, $p > 0.1$). The occurrence of place cells is treated in the text.

reduce uncertainty among wave forms with similar appearance, we only used cells whose peak-to-peak waveform amplitude was greater than $150 \mu V$.

Place cell firing was assessed in four 16 min recording sessions grouped into two pairs. The first pair of sessions was separated by 2–3 min, during which time the recording cable was untwisted. After the first two sessions, the mouse was moved from the recording chamber to its home cage for 1–2 hr. The mouse was then returned to the chamber for sessions 3 and 4, also separated by a 2–3 min break in which the cable was again untwisted.

Twenty-six pyramidal cells were recorded from the CA1 region of seven wild-type mice, and 52 pyramidal cells were recorded from the CA1 region in ten CaMKII-Asp286 transgenic mice. For a cell to be called a pyramidal cell, it had to generate complex-spike events. In addition, it had to show long periods of silence, with some interspike intervals > 1.0 sec (Ranck, 1973).

The initial classification of positional firing patterns was done by inspection of color-coded firing-rate maps such as those shown in Figure 3. For each cell, a separate decision about its class was made for each of the four sessions. The primary classification was between place cells and nonplace cells. An example of a place cell is shown in Figure 3 (upper left). To qualify as a place cell, the unit had to show clear location-specific firing confined to one or two fields. Nonplace cells were considered "silent" if they fired only a few action potentials during a session and "noisy" if their average rate was more than about 1 spike/sec without a firing field (Figure 3), but these two categories of nonplace cells were not considered further. (See Experimental Procedures for criteria used for distinguishing place cells from nonplace cells.)

Place Cells Are Less Common and Less Precise in CaMKII-Asp286 Transgenic Mice

We first asked: Do wild-type and CaMKII-Asp286 transgenic mice show a similar number of place cells in the

CA1 region of the hippocampus. We found that about 58% of the cells from wild-type mice and 31% of the cells from transgenic mice were place cells. The probability that these proportions are equal is about 0.026, suggesting that place cells are less frequent in transgenic mice. The difference between the proportions is calculated as a z-score with correction for continuity (CRC Standard Mathematical Tables, 27th Ed., CRC Press, 1984, p. 513).

In addition to being somewhat rarer, the positional firing patterns of place cells in transgenic mice appear to be weaker than those in wild-type mice. This gives the place fields from transgenic animals a less compact appearance than those of wild types. Differences in the precision of spatial firing patterns are visible in Figure 4, in which a representative rate map for each of the 15 wild-type cells (Figure 4A) and 16 transgenic place cells (Figure 4B) is shown.

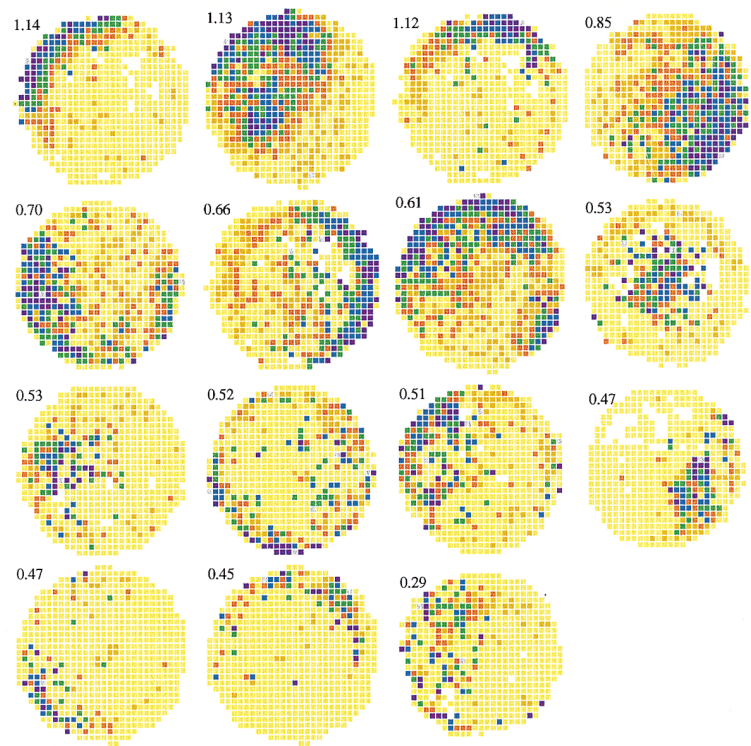
Histograms of coherence for wild-type place cells and transgenic place cells are shown in Figure 5C. Coherence is a nearest-neighbor, two-dimensional autocorrelation that measures the local smoothness of the positional firing pattern. Coherence, as well as the rotational cross-correlation profile we shall consider below, is a correlation method and, as such, is insensitive to absolute firing rates; if all of the rates for either wild-type or transgenic cells were scaled by a multiplicative constant, the coherence values and rotational cross-correlation analysis would be unchanged.

As suggested by the visual appearance of the two-dimensional positional firing patterns and the histograms, a t-test indicates that the mean coherence for wild-type cells (0.621) is higher than that of transgenic cells (0.425) ($t = 2.68$; $df = 29$; $p < 0.012$). This difference does not depend on the three cells with coherence > 1.0 . If these values are eliminated, a t-test shows that the revised mean value for the wild-type cells (0.51) is still reliably greater than the mean for the transgenic cells ($t = 2.28$; $df = 26$; $p < 0.035$).

Other differences exist between the positional firing

A

Firing Rate Maps and Coherence Values for Wild-Type Mice



B

Firing Rate Maps and Coherence Values for CamKII-Asp286 Mice

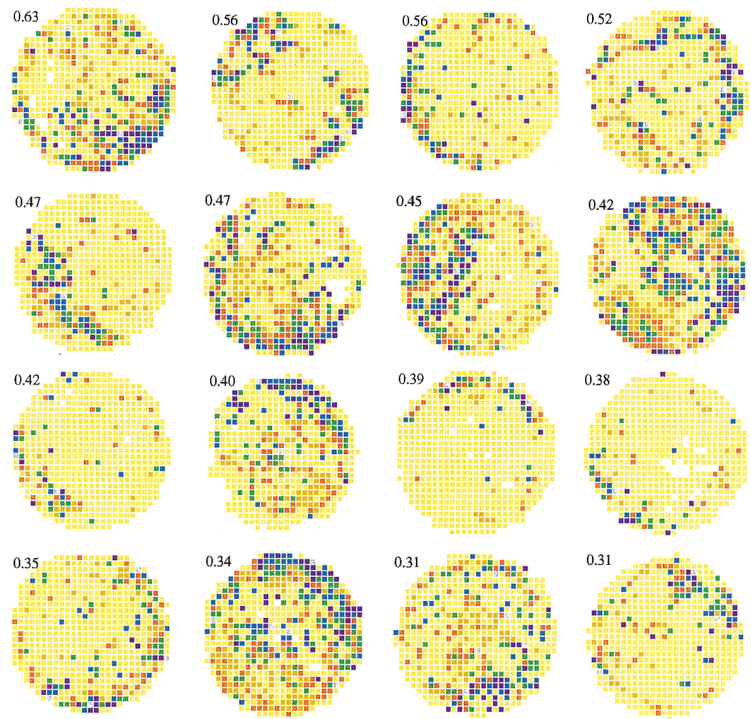


Figure 4. Characteristics of Place Cells in Wild-Type and CaMKII Transgenic Mice
(A) An example of a firing-rate map for each of the 15 cells judged to be place cells in wild-type mice. The rate map is the session with the second highest coherence for the 4 recording sessions. The maps are arranged in descending order of coherence from upper left to lower right. The coherence value for each positional firing pattern is shown to the upper left of each map.
(B) The firing-rate map for each of the 16 mutant place cells from the session with the second highest coherence. Again, the maps are arranged in descending coherence order. By visual inspection, it seems clear that the firing fields of wild-type place cells are crisper than those of transgenics.

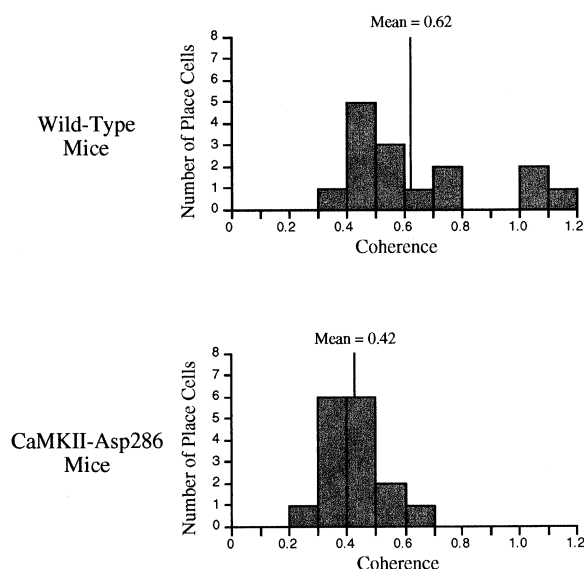


Figure 5. Histograms of the Coherence for 15 Wild-Type and 16 Transgenic Place Cells

The coherence values are averages over 4 sessions for all wild-type cells and 11/16 transgenic cells. For the remaining 5 transgenic cells, the averages are taken over only the 3 sessions in which the positional firing pattern was judged to be characteristic of a place cell.

patterns of wild-type and transgenic cells. The average firing rate of wild-type cells (1.46 spikes/sec) is higher than for transgenic cells (0.52 spikes/sec) ($t = 2.33$; $df = 29$; $p < 0.03$). The peak firing rate of wild-type cells (27.2 spikes/sec) is also higher than for transgenic cells (16.1 spikes/sec) ($t = 2.25$; $df = 29$; $p < 0.04$). These two measures therefore indicate that the transgenic cells discharge more slowly than the wild-type, but the peak firing rate for the transgenic cells is quite reasonable.

It is possible that as a result of the relatively high average rate, the number of pixels in which spikes were detected ("spike area") is slightly, but not reliably, greater for wild-type cells (224 pixels) than for transgenic cells (167 pixels) ($t = 1.55$; $df = 29$; $p > 0.1$). When spike area is normalized by firing rate, however, the discharge of the wild-type cells is more tightly restricted (251 pixels) than for transgenic cells (410 pixels) ($t = 3.37$; $df = 29$; $p < 0.003$). In line with conclusions drawn from coherence, the greater dispersion of action potentials over the apparatus surface suggests that the firing fields of transgenic cells are less precise than that of wild-type cells.

Place Fields in CAMKII-Asp286 Mice Are Unstable

The previous results demonstrate that while the CaMKII-Asp286 mice have place cells, their place fields are less precise. The presence of place cells in the CaMKII-Asp286 mice indicates that the transgenic mice still have the capacity to encode spatial information. Yet their performance on spatial memory tasks is severely impaired. We therefore next asked whether the spatial information contained in the firing pattern of the place cells of the transgenic animals is maintained over time, as would be required for normal performance on spatial

memory tasks. In wild-type rats, the place field of a given neuron is stable over a period of weeks to months (Thompson and Best, 1990).

To assess the stability of place cells in wild-type mice, we used four separate recording sessions. Figures 6A and 6B illustrate the range of stability for wild-type and transgenic place cells, respectively. As this figure illustrates, in wild-type mice the place cells are stable; their fields remain in approximately the same position across the four recording sessions. By contrast, the positional firing patterns of transgenic place cells are generally less stable than those of wild-type place cells.

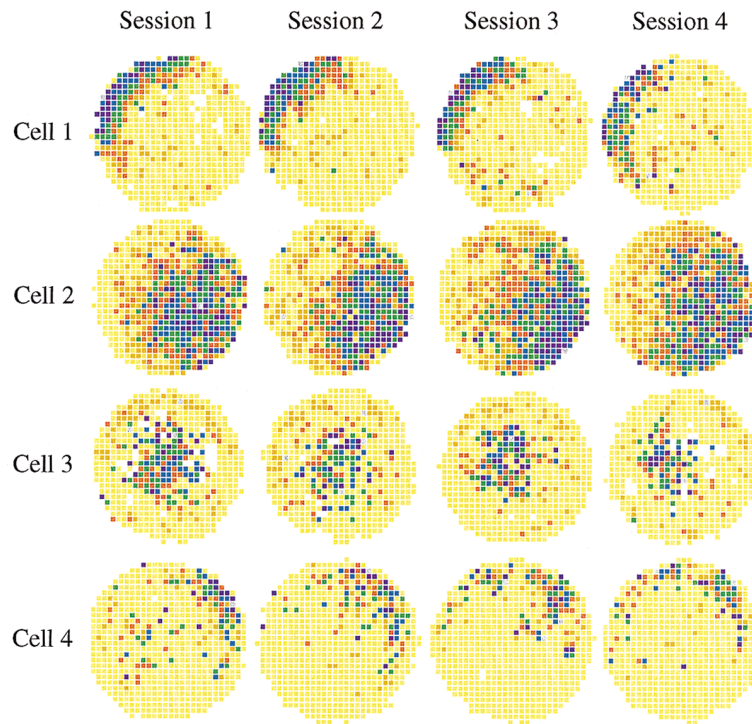
The relative instability of place cells in the transgenic animals can be quantified by computing the rotational cross-correlation profile for pairs of recording sessions. In this method, the positional firing rate maps from two sessions are compared by rotating one of the maps in one-degree increments in relation to the other and calculating a correlation coefficient between the two maps at each increment. From this analysis, we derive two parameters: (1) the angular displacement required to produce the greatest correlation and (2) the magnitude of the maximal correlation. We refer to the maximal correlation as the "similarity" score. An ideal place cell would generate identical firing-rate profiles in each pair of sessions, so that the similarity score would be 1.0 and the required angular displacement would be 0° . If there are four recording sessions, the maximal correlation coefficient and angular displacement are calculated for all six session pairs, and the value for the cell is the average across the six session-pair values. In some cases, the place cell was silent in one of the four sessions. If only three sessions are used for cells, the averages are calculated for only three session pairs.

Histograms of similarity values and angular displacements for wild-type and transgenic place cells are shown in Figure 7. A t-test reveals that the mean similarity for the wild-type place cells (0.54) is higher than the mean for transgenic cells (0.34) [$t = 4.30$; $p(t > 4.30) = 0.00016$]. A second t-test confirms that the mean angular displacement for wild-type cells (20.3°) is less than that for transgenic cells (45.5°) ($t = 2.53$; $p(t > 2.53) = 0.019$). Thus, the transgenic place cells produce sequential firing-rate patterns that bear less resemblance to each other and that do not occur as reliably in a particular part of the cylinder. Together, these findings indicate that transgenic place cells are less stable than wild-type cells.

The lower mean firing rates for transgenic cells could, in principle, reduce the stability of transgenic place cells. If this were the case, cells with lower firing rates should have higher angular displacements and lower similarities. We find, however, that there is little correlation between the mean firing rate and the angular displacement ($r = -0.147$; $r^2 = 0.022$). Therefore, the firing rate accounts for a very small fraction of the variance of the angular displacement. Similarly, the correlation between mean firing rate and similarity is very low ($r = 0.083$; $r^2 = 0.0069$). The lack of correlation between mean firing rate and stability is visible in Figure 6B, where the most stable transgenic place cell (Cell 1) has a lower rate than two of the less stable examples (Cell 2 and Cell 3). The substantial average peak firing rate for transgenic place

A

Place Cell Stability: Wild-Type Mice



B

Place Cell Stability: CaMKII-Asp286 Mice

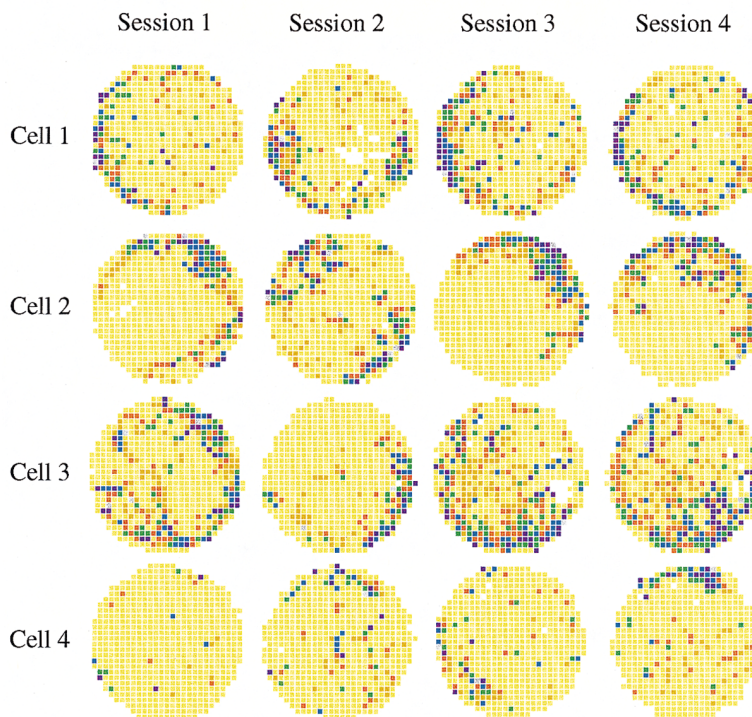


Figure 6. Stability of Place-Cell Firing Patterns

(A) Wild-type mice. Each row shows the rate map from four successive recording sessions for a single place cell from a different mouse. The top row summarizes the activity of a cell with very high mean coherence (1.04), a high mean similarity score (0.75), and a small mean angular displacement between sessions (7.3°). The second row is for a cell with a very large field; the mean coherence was 0.75, the mean similarity was 0.72, and the mean angular displacement was 1.2° . A cell with a central field is shown in the third row; the mean coherence was 0.51, mean similarity 0.64, and mean angular displacement 56° . The mean angular displacement is large because the field was so near the cylinder center that small shifts in the positional firing distribution could yield large changes in the angular field position. The bottom row is for a cell whose mean coherence (0.4) is lower than the average for wild-type units (0.62), whose mean similarity (0.41) is lower than the sample mean (0.54), and whose mean angular displacement (32°) is larger than the sample mean (20.3°). Nevertheless, by inspection and from numerical comparisons, the positional firing pattern reproducibility for this cell is about equal to the best mutant place cell (see below).

(B) Transgenic mice. The top row shows the transgenic place cell with the most reproducible positional firing pattern. The mean coherence was 0.47, mean similarity 0.49, mean angular displacement 68° , and mean firing rate 0.43 spikes/sec. Note that the firing pattern was stable even though the firing rate was lower than the average for transgenic place cells. The second row shows a transgenic place cell whose positional firing pattern was reproducible for sessions 1, 3, and 4. The pattern in session 2, however, bears a scant resemblance to the others. The mean coherence for this cell was 0.61, mean similarity 0.41, mean angular displacement 30° and mean firing rate 1.2 spikes/sec. This cell showed instability even though its mean firing rate was not far from the average for wild-type cells. The third row shows rate maps for a typical mutant place cell. The mean coherence was 0.45, mean similarity 0.36, mean angular displacement 39° , and mean firing rate 0.65. The mean firing rate is well within the range observed for wild-type place cells. The bottom row is for a cell that was silent in the first session and showed a weak field at 12:00 o'clock in session 2, a stronger field at 7:00 o'clock in session 3, and a fairly crisp field at 12:30 o'clock in session 4. Means for this cell were taken over the last three sessions only. The mean coherence was 0.51, mean similarity 0.28, mean angular displacement 86° , and mean firing rate 0.15 spikes/sec. This is a low rate, but the firing density clearly moved in sessions 2, 3 and 4.

cells (16.1 spikes/sec) provides a third indication that reduced firing rate is not the primary cause of instability.

In a second analysis of place-cell stability in transgenics, we compared similarity and angular displacement for session pairs that were separated by short (2–3 min) intervals and session pairs separated by longer (1–2 hr) intervals. For each of the 11 transgenic cells judged to be a place cell in all four sessions, similarity and angular displacement were calculated for session pair 1 and 2 and pair 3 and 4 for short intervals and for session pair 1 and 3 and pair 2 and 4 for long intervals. The means for short and long intervals were used to compare stability on the two time scales. Interestingly, there were no differences in either measure. The mean similarity was 0.36 for short intervals and 0.38 for longer intervals (paired $t = 1.10$; $df = 10$; $p > 0.25$). The mean angular displacement was 43.5° for short intervals and 34.3° for longer intervals (paired $t = 1.15$; $df = 10$; $p > 0.25$). We conclude that there is no tendency for the firing patterns in closely spaced sessions to resemble each other more than the patterns in sessions separated more in time.

Discussion

The studies outlined here represent a first step in a molecular analysis of how a spatial map is constructed. Previously, we examined Schaffer collateral LTP at the hippocampal CA1 synapse in a line of transgenic mice that expresses a constitutively active form of CaMKII. In these mice, normal LTP is produced by stimulation at high frequencies (100 Hz), whereas a deficit in LTP is found in response to stimulation in the 5–10 Hz theta-frequency range. These lower frequencies are the frequency range of naturally occurring oscillations in the EEG (known as the theta rhythm) that are generated when rodents locomote. We now have recorded from place cells in the CA1 region in these mice and addressed three questions: (1) What are the molecular

mechanisms whereby an animal forms a cognitive map in a new environment? (2) Once a cognitive map is formed, does LTP play any role in its maintenance? and, (3) Do place cells contribute to the learning and performance of spatial problems?

Place Cells and Cognitive Maps

Animals gain information about the external world with their five senses. For each sense, there is a representation within the brain in the form of a topographic map of the receptive sheet. For example, mammals have several maps of the body surface in their somatic sensory cortices and numerous maps of the retina in their visual cortices. But in addition to the immediate sensory information, the brain must represent at least two more abstract features of the world that have no receptive sheets: space and time. Place cells provide the first insight into the representation of space.

In rodents such as rats and mice, where an essential part of the representation of space is localized to the hippocampus, the pyramidal cells of the CA1 and CA3 region have place fields. The remarkable discovery of O'Keefe and his colleagues is that each cell fires only when the head of the rat is in a part of space and so encodes location within the environment. O'Keefe (1976) gave these cells the name "place cells" and, by analogy to sensory receptive fields, called the cell-specific region of intense activity the "place field." Place cells behave in a fashion that resembles sensory cells. However, the similarity between place cells and, for instance, visual cortical neurons is only superficial and breaks down in two fundamental ways.

First, the map of the environment is not topographic. Unlike sensory maps, neighboring place cells in the hippocampus do not have neighboring place fields in the environment (Muller et al., 1987; Kubie et al., 1992), although there may be a slight tendency for clustering (Eichenbaum et al., 1989). This raises the question: Why should the organization of the spatial representation in

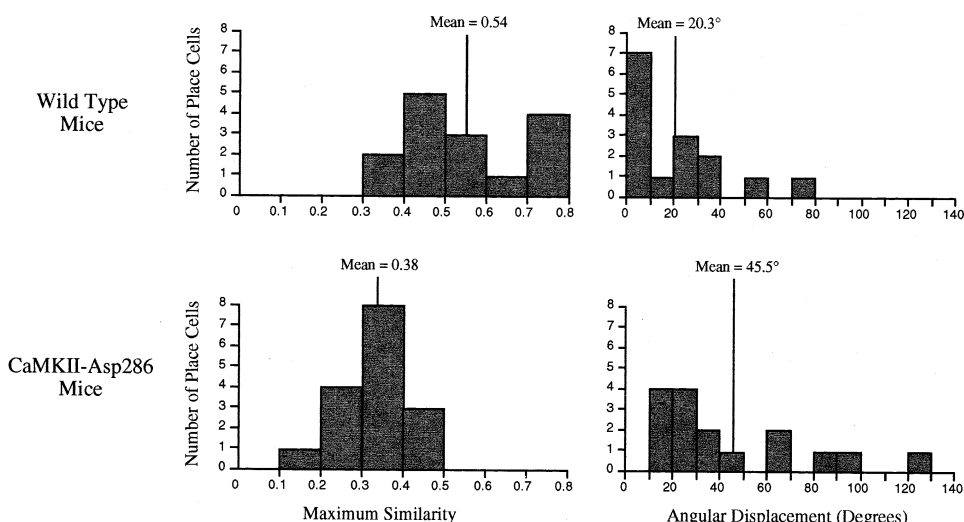


Figure 7. Histograms of the Distributions of Maximum Similarity and Angular Displacement for Wild-Type and Transgenic Place Cells. As confirmed by t -tests (see text), the similarity scores are higher and the angular displacements lower for wild-type cells.

the hippocampus be so different from sensory representations in neocortex? One possibility is that the hippocampus does not signal the stimulus pattern falling on a sensory receptive sheet, a sheet that has a relatively fixed set of connections from the periphery to the cortex. Rather, the hippocampus signals the position of the animal within the environment, and there is no constant relationship between the environmental surface and the hippocampal surface because different environments differ so dramatically in size and shape. Instead of a topographic map of the environment, the hippocampus may provide a topological map (Muller et al., 1996). In a topographic map, distance in the environment would be represented by distance between cells in the cell layer; in a topological map, distance in the environment might be represented by the strength of synaptic connections between pairs of place cells regardless of their location in the cell layer.

Second, the analogy between place cells and sensory cells breaks down when the relationship between cell activity and sensory stimuli is examined. For instance, in rats and mice, rotating the primary visual cue in the experimental environment that we used, the white cue card (Figure 1), causes place fields to rotate equally. Thus, the card has a powerful form of stimulus control over place cells. If place cells are properly thought of as sensory cells, removal of the card should disrupt place-cell firing. In fact, removal of the card leaves place fields intact, although they may rotate by an unpredictable amount inside the cylinder (Muller and Kubie, 1987; Rotenberg and Muller, unpublished data).

Thus, the hippocampal map of space appears to be a cognitive map, not a sensory map. The activity of its cellular components represents not specific sensory input but an abstract feature of the world: space. One central feature of the spatial representation in the hippocampus is that individual pyramidal cells participate not in one, but in multiple maps. This flexible reuse of cells presumably reflects changes in synaptic strength and raises the question of how the maintenance of multiple maps corresponds to the ability to learn to solve spatial problems in multiple environments.

Synaptic Plasticity, Place Cell Properties, and Cognitive Maps

As a first step in relating the biology of place fields to that of synaptic plasticity, we have used genetically modified mice that have a selective defect in a defined component of LTP and a defect in a defined component of spatial learning. We therefore have tested the idea that the LTP defect might disrupt the hippocampal cognitive map, rendering the mice incapable of solving spatial problems. Since place cells are thought to be the foundation of the map on the systems level, we examined the possibility that place cells in the CaMKII-Asp286 transgenic mice would differ in detectable ways from place cells in wild-type mice.

Analysis of place cells in the CA1 region of CaMKII-Asp286 mice reveals four differences from wild-type mice. First, the number of place cells was lower in the transgenic animals. Second, the average firing rate of the neurons in the CaMKII-Asp286 mice was slower relative to wild-types. Third, the place fields were less well-defined in the transgenic mice. Finally, the place cells

that formed in the CaMKII-Asp286 transgenic mice were unstable between recording sessions separated by only a few minutes.

Instability of Place Fields and Instability of Spatial Memory

Taken together, the data on LTP, place-cell activity, and spatial problem-solving ability in the CaMKII-Asp286 mice shed light on the molecular and cell-biological building blocks of spatial cognition. Previous results demonstrate the CaMKII-Asp 286 transgenic mice lack LTP in the 5–10 Hz range and are severely impaired on a spatial task, a task that requires navigation to an unmarked goal, but retain the ability to solve the nonspatial versions of the same task in which the animal is required to go to a marked goal (Bach et al., 1995; Mayford et al., 1995). The current study demonstrates that in the CA1 region of the hippocampus the place cells are less abundant, less well-defined, and less stable over time. How is the deficit in LTP in the 5–10 Hz range reflected in the deficits in the properties of place cells? In turn, how might the defects in place-cell firing lead to the observed behavioral deficits?

That place cells do actually form in CaMKII-Asp286 mice indicates that these animals can transform sensory information into spatial information. Thus, synaptic strengthening caused by activity in the 5–10 Hz range is not necessary for the formation of place cells. However, synaptic strengthening in this low-frequency range may be required for some aspect of the maintenance or stabilization of the place cell. As shown in Figure 2B, these transgenic mice have normal theta-frequency EEG activity during locomotion, even though they lack the ability to strengthen synaptic connections in response to activity in this frequency range (Mayford et al., 1995). Perhaps the inability to strengthen synaptic connections during theta-frequency cell discharge leads to the reduction in the number, precision, and stability of place-field firing patterns.

In parallel, the deficits in place-cell activity have features that might allow them to account for the behavioral deficit. For example, the decreased fraction of place cells, the lower firing rate of place cells, and the more dispersed positional firing evident in the transgenic mice might alter the hippocampal network sufficiently to make solving the spatial navigational problem inefficient or impossible. To determine if such quantitative differences in place-cell activity are sufficient to account for the difficulties in spatial problem solving will require detailed computational models.

Although these quantitative differences are interesting, the dramatic, qualitative differences in the stability of place cells over time provide a more compelling basis for understanding a component of the behavioral deficits of the transgenic mice. In wild-type mice, the firing fields are constant over repeated exposures of the animal to a familiar environment. This constancy requires a form of memory and is consistent with the idea that the mouse has stored information about the layout of its environment in a cognitive map. Presumably, this mapping information is then used to solve spatial navigation tasks. The inability to maintain stable place cells

in the CaMKII-Asp 286 transgenic mice implies instability in the cognitive map and, therefore, an inability to use the map to solve spatial navigation tasks. Moreover, the ability of the CaMKII-Asp286 transgenics to solve marked-goal navigational problems is consistent with the presence of place cells in these animals and implies an ability to encode an immediate representation of their environment, even though the representation cannot be maintained over time.

Attention, Distraction, and the Stability of Place Fields

Why are transgenic place cells reasonably stable within 16 min sessions, and why are they often unstable between pairs of sessions? A possible clue comes from the observation that the positional firing patterns for session pairs separated by one or two hours do not differ from each other more than the patterns from sessions separated by only two or three minutes. There is no evidence for a continuous decay of memory. Instead, the changes seem to be equal in magnitude regardless of the interval between session pairs, at least in the limited range of intervals we used here.

One explanation of the equal disruption by both short as well as long intersession intervals is that firing-field instability might be caused by any disruption of the ongoing activity of the mouse. According to this view, the time during which transgenic place cells are stable is determined by the interval during which the environment is stable and the attention of the animal is not distracted. Once the environment changes and the animal is distracted, individual place cells, and therefore the map, become unstable. According to this view, even the brief interruption between closely spaced recording sessions is a sufficient disruption of the environment of the mouse to cause the place cells to lose stability.

This suggestion draws attention to an interesting parallel between the properties of place cells in CaMKII-Asp286 transgenic mice and memory function in humans who are amnesic because of hippocampal damage. Such people have fairly stable memories for explicit events over short time intervals and unstable memories over longer intervals. What is striking is that any abrupt change in the environment of an amnesic person can cause very rapid forgetting. If a newly introduced stranger leaves the presence of the amnesic person for a short interval and then returns, he will not be recognized. In contrast, if the stranger stays continuously in the presence of the amnesic person, he is treated as familiar.

This idea leads to two experimental tests. First, stability or instability of place fields should be seen over the same wide range of time spans, depending on whether the mouse is left alone or disturbed. Second, the CaMKII-Asp286 transgenic mice may be able to learn a spatial task that can be acquired in a single, continuous training session. If so, they will show no evidence of savings if they are removed and then returned to the experimental chamber. As these two tests indicate, the ability to apply genetic manipulation with research on cognitive maps permits a new level of analysis in the study of memory.

LTP, Place-Cell Stability, and Spatial Memory

Do the deficits in LTP that we have observed account for all of the impairments in place-cell properties, and are these impairments responsible for all of the memory deficits? These questions are not fully addressed by our studies. We have used a single genetic manipulation to try to establish a link between two physiological processes, LTP in the theta-frequency range and place-field stability, and this leads to results that are, by definition, correlative. The expression of the CaMKII-Asp286 transgene clearly disrupts both LTP and place-cell stability. However, this does not necessarily imply a common underlying mechanism. Of particular concern with genetically modified mice is that the expression of the transgene may produce secondary developmental defects and these defects might lead to the observed phenotype in the adult animal. We have recently shown, using regulated transgene expression, that suppression of the CaMKII-Asp286 in adult animals reverses both the theta-frequency LTP deficit and the spatial-learning impairment (Mayford et al., 1996). These results demonstrate that any developmental effects that might be produced by CaMKII-Asp286 expression do not affect the physiology of the adult hippocampus in the slice, nor do they affect behavioral memory in the intact animal. Thus, the observed phenotypes in the adult animal are likely to reflect the acute effect of the transgene on CaMKII activity. These results, with regulated expression of CaMKII-Asp286, strengthen the correlation between the various phenotypic measures (LTP, place cells, and spatial memory) by indicating that all three are likely to be consequences of an acute effect of the transgene on the signaling capabilities of CaMKII in the adult animal.

Does the effect of the transgene on the synapse between Schaffer collaterals and CA1 pyramidal cells account for the place-field deficit, or do deficits in other circuits, e.g., entorhinal or perirhinal cortices, contribute to the place-cell deficits? We specifically chose, for the present study, to analyze the P15 line of CaMKII-Asp286 mice. This line shows strong transgene expression in the hippocampus and a much weaker expression in other brain regions (Mayford et al., 1995). It is therefore likely that the place-cell deficits result from the alterations in the hippocampus. However, there is no reason to believe that within the hippocampus the deficit is restricted to the Schaffer collateral synapse onto the CA1 pyramidal cells. A conclusive determination of which hippocampal site is involved must await more restricted expression of the transgene specifically to the CA1 neurons as described in Tsien et al. (1996 [this issue of *Cell*]).

Experimental Procedures

The general methods are modifications of methods previously used to record place cells in rats (Muller et al., 1987). Place cells were recorded as mice ran around in a seamless, gray plywood cylinder (49 cm diameter, 34 cm height). The cylinder was surrounded by a 2 m diameter cylindrical curtain centered in a 2.6 × 2.6 m recording room. A white cardboard card inside the cylinder cover 90° of arc. The floor was lined with gray paper that could be replaced. Four 25 W bulbs in overhead reflectors provided even illumination of the cylinder floor. Mice ran around eating pulverized food pellets scattered on the cylinder floor. The mice appeared able to pick up food particles and eat them without pausing and ran almost continuously.

Recordings were made with an electrode array modified from a design for rats (Kubie, 1984). The new electrode array was smaller and lighter than that used for rats. To build the array, 27 insulated 25 mm nichrome electrode wires were threaded through two 26-gauge stainless steel cannulas. Mice were anesthetized with 58 mg/kg sodium pentobarbital given IP. After they were unconscious, they were placed in a mouse stereotaxic instrument. Electrode implantation was done under sterile conditions. The initial placement for the electrode tips was 2.0 mm anterior to bregma, 2.0 mm lateral to midline, and 1.0 mm below dura, directly above the dorsal hippocampus. The electrode array was secured to the skull with Grip Cement (Dentsply International Inc.). Bacitracin ointment was applied to the tissue surrounding the electrode, and the mice were given one week to recover before starting experiments. Grip Cement polymerization is sufficiently exothermic that the heat generated may damage dorsal neocortex in mice. Two precautions were taken to avoid such damage. First, the applied cement was in contact with a brass nose clamp that served as a heat sink. Second, the cement was applied in thin layers to facilitate heat dissipation. Mice were given several days to recover after surgery before recordings were attempted.

To look for discriminable single-cell electrical activity, each microwire electrode was screened with the mouse in the recording cylinder. If no usable units were detected, the entire electrode array was advanced in steps of 20 or 40 μm and the mouse was returned to its home cage for an hour or more to allow the electrodes to settle. The screening sequence was repeated until one or more pyramidal cells (identified as complex-spike cells; see Figure 2A) could be isolated. To minimize uncertainty and sampling bias, a cell was always used if the peak-to-peak amplitude of its waveform was greater than 150 μV .

Each cell or group of simultaneously detected cells was recorded for 64 min in two pairs of 16 min sessions. The first pair of sessions was separated by a 2–3 min break during which the experimenter entered the recording room to untwist the recording cable (see Figure 1) so that the mouse could continue to move freely. After the second session, the mouse was taken out of the recording chamber and moved to its home cage for 1–2 hr. It was then returned to the chamber for sessions 3 and 4, which were also separated by a 2–3 min break to untwist the cable. Amplified signals ($\times 10000$) were filtered between 300 Hz and 10 KHz for unit recordings and between 0.3 and 300 Hz for EEG recordings. Waveforms were sampled with a Datawaves workstation in 800 μs bursts at 40 KHz. The waveforms before the mouse was removed from the cylinder and after it was returned to the cylinder were in general indistinguishable; cells were rarely, if ever, lost due to unplugging and replugging the cable connector.

The head position of the mouse was tracked at 60 Hz with an overhead TV camera that detected a light-emitting diode (LED) melted on the electrode connector. The LED was detected in a 64×64 array of small square regions (pixels) 1.6 cm on each side. The total time the light was detected in each pixel and the number of spikes fired in each pixel were accumulated, and a time-averaged firing rate was calculated for each pixel by dividing spike count by dwell time. Color-coded firing-rate maps are used to visualize positional firing-rate distributions. Pixel rates were sorted in ascending order, partitioned into six categories, and coded in the sequence: yellow, orange, red, green, blue and purple. In yellow pixels the firing rate was exactly zero. The lowest nonzero rates are encoded orange; the highest nonzero rates, purple. Unvisited pixels in the cylinder and pixels outside the cylinder are coded white.

Several quantitative measures were used to describe and compare the properties of positional firing patterns in wild-type and transgenic mice. First, we estimated place-field area. A field is a group of contiguous pixels such that a pixel is included in the field if its firing rate is greater than zero and it shares an edge with another pixel already part of the field. Second, we computed coherence. To find the coherence of a positional firing pattern, a list is made of the firing rate in each pixel in the apparatus. For each pixel, the firing rate averaged over the nearest neighbors to the pixel is also calculated. The correlation coefficient between the rate and the nearest neighbor rate is calculated. Coherence is the z-transform of the correlation. It is an estimate of the local smoothness of the positional firing pattern since it describes the ability of the nearest

neighbors to predict the firing rate in a central pixel. Coherence has been used to contrast the precision of positional firing patterns of different cell classes (Kubie et al., 1990; Quirk et al., 1992) and for other purposes (Muller and Kubie, 1989; Sharp et al., 1995; Taube, 1995). We also calculated peak firing rate by maximizing the firing rate in each 3×3 group of contiguous pixels.

To objectively demonstrate differences between place cells and nonplace cells, we combined field area and coherence values. We adjusted field size and coherence criteria until all 15 wild-type cells judged by visual inspection to be place cells were chosen. To do this, the coherence was required to be greater than 0.26, and the area of the largest field had to be at least 10 pixels but less than 70% of the apparatus area. These criteria selected all 15 wild-type place cells and an additional cell judged to be noisy (see Figure 3). When the same criteria were applied to transgenics, the place cells selected by the algorithm were exactly the same as those previously called place cells from visual inspection.

Numerical estimates of the constancy of place-cell firing across session pairs were made by computing a rotational cross-correlation profile. To begin, the correlation coefficient is calculated on a pixel-by-pixel basis for a pair of positional firing-rate patterns. One pattern is then rotated in 360 one-degree steps around the cylinder center, and at each step the correlation is recalculated. The highest correlation in the 360 values is found. The magnitude of this correlation estimates the similarity of the two positional firing patterns. The angle at which the maximum correlation is found estimates the extent to which one positional firing pattern is rotated with respect to the other.

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